

In the Claims

The listing of claims will replace all prior versions and listing, of claims in the application:

Listing of Claims:

1. (Original) A recombinant polynucleotide, the polynucleotide comprising a first and a second sequence, the first sequence encoding a signal peptide comprising a TAT signal and a Sec avoidance signal and the second sequence encoding a heterologous protein, wherein the sequence of the signal peptide is

M-X₁-K/R-X₂-K/R-X₃-RR-X₄-K/R-A

in which X₁ is a sequence of 0 to 10 amino acids;
X₂ is a sequence of 0 to 3 amino acids;
X₃ is a sequence of 0 to 10 amino acids; and
X₄ is a sequence of 15 to 24 amino acids in which at least 75% up to about 90% of the residues are hydrophobic.

2. (Original) A recombinant polynucleotide according to claim 1 wherein X₁ is a sequence of 0 to 5 amino acids, and is preferably 0.
3. (Currently Amended) A recombinant polynucleotide according to claim 1 ~~or 2~~ wherein X₂ is a sequence of 0 or 1 amino acid, preferably 0.
4. (Currently Amended) A recombinant polynucleotide according to ~~any one of claims 1 to 3~~ claim 1 wherein X₃ is a sequence of 0 to 5 amino acids, preferably 0.
5. (Currently Amended) A recombinant polynucleotide according to ~~any one of claims 1 to 4~~ claim 1 wherein X₄ is a sequence of at least 20 amino acids of which at least 18 are hydrophobic amino acids.

6. (Currently Amended) A recombinant polynucleotide according to ~~any one of claims 1 to 5~~ claim 1 wherein X₄ is 23 amino acids.
7. (Currently Amended) A recombinant polynucleotide according to ~~any one of claims 1 to 6~~ claim 1 wherein the sequence of the signal peptide is MKKRRVVNSVLLLLLLASALALTVPMAKA (SEQ ID NO:1).
8. (Original) A signal peptide, the signal having the sequence

M-X₁-K/R-X₂-K/R-X₃-RR-X₄-K/R-A.

- in which X₁ is a sequence of 0 to 10 amino acids;
X₂ is a sequence of 0 to 3 amino acids;
X₃ is a sequence of 0 to 10 amino acids; and
X₄ is a sequence of 15 to 24 amino acids in which at least 75% up to about 90% of the residues are hydrophobic.
9. (Original) A signal peptide according to claim 8 wherein X₁ is a sequence of 0 to 5 amino acids, and is preferably 0.
 10. (Currently Amended) A signal peptide according to claim 8 ~~or 9~~ wherein X₂ is a sequence of 0 to 1 amino acid, preferably 0.
 11. (Currently Amended) A signal peptide according to ~~any one of claims 8 to 10~~ claim 8 wherein X₃ is a sequence of 0 to 5 amino acids, preferably 0.
 12. (Currently Amended) A signal peptide according to ~~any one of claims 8 to 11~~ claim 8 wherein X₄ is a sequence of at least 20 amino acids of which at least 18 are hydrophobic amino acids.

13. (Currently Amended) A signal peptide according to ~~any one of claims 8 to 12~~ claim 8 wherein X_4 is 23 amino acids.
14. (Currently Amended) A signal peptide according to ~~any one of claims 8 to 13~~ claim 8 wherein the sequence of the signal peptide is MKRRRVVNSVLLLLLLASALALTVPMAKA (SEQ ID NO 1).
15. (Original) A method of producing a heterologous polypeptide from a host cell comprising a TAT translocation system, the method comprising:

(i) transforming the host cell with a DNA sequence encoding the heterologous polypeptide and a signal peptide wherein the signal peptide comprises a TAT signal and a Sec avoidance signal wherein the sequence of the signal peptide is

$$M-X_1-K/R-X_2-K/R-X_3-RR-X_4-K/R-A$$

in which X_1 is a sequence of 0 to 10 amino acids;
 X_2 is a sequence of 0 to 3 amino acids;
 X_3 is a sequence of 0 to 10 amino acids; and
 X_4 is a sequence of 15 to 24 amino acids in which at least 75% up to about 90% of the residues are hydrophobic.

(ii) culturing the host cell under conditions which allow expression of the heterologous polypeptide; and

(iii) recovering the heterologous polypeptide secreted from the host cell via the TAT translocation system.

16. (Original) A method according to claim 15 wherein X_1 is sequence of 0 to 5 amino acids, and is preferably 0.

17. (Currently Amended) A method according to claim 15 ~~or 16~~ wherein X_2 is a sequence of 0 or 1 amino acid, preferably 0.
18. (Currently Amended) A method according to ~~any one of~~ ~~claims 15 to 17~~ claim 15 wherein X_3 is a sequence of 0 to 5 amino acids, preferably 0.
19. (Currently Amended) A method according to ~~any one of~~ ~~claims 15 to 18~~ claim 15 wherein X_4 is a sequence of at least 20 amino acids of which at least 18 are hydrophobic amino acids.
20. (Currently Amended) A method according to ~~any one of~~ ~~claims 15 to 19~~ claim 15 wherein X_4 is 23 amino acids.
21. (Currently Amended) A method according to ~~any one of~~ ~~claims 15 to 20~~ claim 15 wherein the sequence of the signal peptide is MKKRRVNSVLLLLLLASALALTVAPMAKA (SEQ ID NO:1).
22. (Currently Amended) A method according to ~~any one of~~ ~~claims 15 to 21~~ claim 15 wherein the host cell is *Bacillus sp.*
23. (Original) A method according to claim 22 wherein the host cell is selected from the group consisting of *Bacillus choshinensis*, *Bacillus brevis*, *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus megatorium*.
24. (Original) A method according to claim 22 wherein the host cell is *Bacillus choshinensis*.
25. (Currently Amended) A method according to ~~any one of~~ ~~claims 15 to 24~~ claim 15 wherein the heterologous polypeptide is a polypeptide which readily folds in the cytoplasm.

26. (Currently Amended) A method according to ~~any one of claims 15 to 25~~ claim 15 wherein the polynucleotide encoding the mature polypeptide has a sequence selected from:
- (i) a sequence of nucleotides shown in SEQ ID NO:29 from nucleotide 85 to 1155;
 - (ii) a sequence that hybridises to SEQ ID NO:29 from nucleotide 85 to 1155 under conditions of high stringency;
 - (iii) a sequence which is greater than 90% identical to SEQ ID NO:29 from nucleotide 85 to 1155; and
 - (iv) a sequence that encodes the amino acid sequence provided in SEQ ID NO:30 from residue 29 to 384.
27. (Currently Amended) A method according to ~~any one of claims 15 to 25~~ claim 15 wherein the mature heterologous polypeptide comprises the sequence provided in SEQ ID NO:30 from residue 29 to 384; or a polypeptide which is greater than 90% identical to the sequence provided in SEQ ID NO:30.
28. (Currently Amended) A substantially purified polypeptide produced according to the method of ~~any of claims 15 to 27~~ claim 15.
29. (Currently Amended) A vector comprising the recombinant polynucleotide according to ~~any of claims 1 to 8~~ claim 1.
30. (Currently Amended) A host cell comprising the recombinant polynucleotide according to ~~any of claims 1 to 8~~ claim 1.